

CLAIMS

1. A substantially pure preparation of a plasmin inhibitor characterised in that it is a single stage competitive inhibitor of plasmin.
2. The plasmin inhibitor of claim 1 further characterised in that it has a  
5 dissociation constant for plasmin in the range of from  $1 \times 10^{-8} \text{ M}^{-1}$  to  $1 \times 10^{-10} \text{ M}^{-1}$ .
3. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation constant for plasmin in the range of from  $5 \times 10^{-8} \text{ M}^{-1}$  to  $8 \times 10^{-9} \text{ M}^{-1}$ .
- 10 4. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation constant for plasmin in the range of from  $1 \times 10^{-9} \text{ M}^{-1}$  to  $5 \times 10^{-9} \text{ M}^{-1}$ .
5. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation rate constant for plasmin in the range of from  $4 \times 10^{-5} \text{ sec}^{-1} \text{ M}^{-1}$  to  
15  $5 \times 10^{-7} \text{ sec}^{-1} \text{ M}^{-1}$ .
6. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation rate constant for plasmin in the range of from  $1 \times 10^{-6} \text{ sec}^{-1} \text{ M}^{-1}$  to  $1 \times 10^{-7} \text{ sec}^{-1} \text{ M}^{-1}$ .
7. The plasmin inhibitor of claim 1 further characterised in that it has a  
20 dissociation rate constant for plasmin in the range of from  $2 \times 10^{-6} \text{ sec}^{-1} \text{ M}^{-1}$  to  $9 \times 10^{-6} \text{ sec}^{-1} \text{ M}^{-1}$ .
8. The plasmin inhibitor of claim 1 comprising a polypeptide selected from the group consisting of:
  - (a). Lys-Asp-Arg-Pro-Asp-Phe-Cys-Glu-Leu-Pro-Ala-Asp-Thr-Gly-Pro-Cys-  
25 Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-Tyr-Asn-Pro-Asp-Glu-Lys-Lys-Cys-  
Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Glu-Gly-Asn-Ala-Asn-Asn-Phe-Ile-  
Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:2];

- (b). Lys-Asp-Arg-Pro-Glu-Leu-Cys-Glu-Leu-Pro-Pro-Asp-Thr-Gly-Pro-Cys-Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-Tyr-Asn-Pro-Asp-Glu-Gln-Lys-Cys-Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Glu-Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:4];
- 5 (c). Lys-Asp-Arg-Pro-Asn-Phe-Cys-Lys-Leu-Pro-Ala-Glu-Thr-Gly-Arg-Cys-Asn-Ala-Lys-Ile-Pro-Arg-Phe-Tyr-Tyr-Asn-Pro-Arg-Gln-His-Gln-Cys-Ile-Glu-Phe-Leu-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Lys-Thr-Ile-Lys-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:6];
- 10 (d). Lys-Asp-His-Pro-Lys-Phe-Cys-Glu-Leu-Pro-Ala-Glu-Thr-Gly-Ser-Cys-Lys-Gly-Asn-Val-Pro-Arg-Phe-Tyr-Tyr-Asn-Ala-Asp-His-His-Gln-Cys-Leu-Lys-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Lys-Thr-Ile-Glu-Glu-Gly-Lys-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:8];
- 15 (e). Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-Leu-Leu-Pro-Asp-Thr-Gly-Ser-Cys-Glu-Asp-Phe-Thr-Gly-Ala-Phe-His-Tyr-Ser-Thr-Arg-Asp-Arg-Glu-Cys-Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:10]; and
- (f). Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-Leu-Pro-Ala-Asp-Ile-Gly-Pro-Trp-Asp-Asp-Phe-Thr-Gly-Ala-Phe-His-Tyr-Ser-Pro-Arg-Glu-His-Glu-Cys-Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Lys-Gly-Asn-Ala-Asn-Asn-Phe-Asn-Thr-Gln-Glu-Gln-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:12];
- 20 (g). a biologically-active fragment of any one of SEQ ID NO:2, 4, 6, 8, 10 and 12; and
- (h). a variant or derivative of any of the foregoing polypeptides or fragments thereof.
- 25 9. The plasmin inhibitor of claim 8 wherein said variant has the general formula:  
 KDZPZYCZLBBZBGXCZXXXBXFÄYXBZZZCBZFBYGGCXBNANNF  
 XTXECESTCAA (I), wherein: -

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- X is any amino acid;  
 Y is a hydrophobic amino acid;  
 A is an aromatic amino acid;  
 Z is K, R, H, D, E, Q or N; and  
 5 B is a neutral amino acid, or P, A, G, S, T, V or L.

10. The plasmin inhibitor of claim 9, wherein the Z at position 3 is H or R.  
 11. The plasmin inhibitor of claim 9, wherein the Z at position 5 is K, N, E or D.  
 12. The plasmin inhibitor of claim 9, wherein the Y at position 6 is F or L.  
 13. The plasmin inhibitor of claim 9, wherein the Z at position 8 is E or K.  
 10 14. The plasmin inhibitor of claim 9, wherein the B at position 10 is P or L.  
 15. The plasmin inhibitor of claim 9, wherein the B at position 11 is P or A.  
 16. The plasmin inhibitor of claim 9, wherein the Z at position 12 is E or D.  
 17. The plasmin inhibitor of claim 9, wherein the B at position 13 is T or I.  
 18. The plasmin inhibitor of claim 9, wherein the X at position 15 is P, S or R.  
 15 19. The plasmin inhibitor of claim 9, wherein the Z at position 17 is K, N, E, D or R.  
 20. The plasmin inhibitor of claim 9, wherein the X at position 18 is D, G, A or V.  
 21. The plasmin inhibitor of claim 9, wherein the X at position 19 is F, N, K or R.  
 22. The plasmin inhibitor of claim 9, wherein the X at position 20 is T, P, F or I.  
 20 23. The plasmin inhibitor of claim 9, wherein the B at position 21 is G, V or P.  
 24. The plasmin inhibitor of claim 9, wherein the X at position 22 is A, S or R.  
 25. The plasmin inhibitor of claim 9, wherein the A at position 24 is Y or H.  
 26. The plasmin inhibitor of claim 9, wherein the X at position 26 is S or N.  
 27. The plasmin inhibitor of claim 9, wherein the B at position 27 is P, A or T.  
 25 28. The plasmin inhibitor of claim 9, wherein the Z at position 28 may be D or R.  
 29. The plasmin inhibitor of claim 9, wherein the Z at position 29 is E, D, H or Q.  
 30. The plasmin inhibitor of claim 9, wherein the Z at position 30 is H, K, R or Q.

31. The plasmin inhibitor of claim 9, wherein the Z at position 31 is K, Q or E.
32. The plasmin inhibitor of claim 9, wherein the B at position 33 is L or I.
33. The plasmin inhibitor of claim 9, wherein the Z at position 34 is E or K.
34. The plasmin inhibitor of claim 9, wherein the B at position 36 is L or I.
- 5 35. The plasmin inhibitor of claim 9, wherein the X at position 41 is E, G or K.
36. The plasmin inhibitor of claim 9, wherein the B at position 42 is C or G.
37. The plasmin inhibitor of claim 9, wherein the X at position 48 is K, N or I.
38. The plasmin inhibitor of claim 9, wherein the X at position 50 is K, Q or I.
39. The plasmin inhibitor of claim 8, wherein the polypeptide comprises a leader  
10 peptide comprising the sequence:- Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-  
Leu-Gly-Leu-Leu-Thr-Leu-Trp-Glu-Val-Leu-Thr-Pro-Val-Ser-Ser [SEQ ID  
NO:14], or a biologically-active fragment thereof, or variant or derivative of  
these.
40. The plasmin inhibitor of claim 39, wherein the polypeptide is selected from  
15 the group consisting of:-
- (a) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-  
Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Asp-Phe-Cys-Glu-  
Leu-Pro-Ala-Asp-Thr-Gly-Pro-Cys-Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-  
Tyr-Asn-Pro-Asp-Glu-Lys-Lys-Cys-Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-  
20 Glu-Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-  
Cys-Ala-Ala [SEQ ID NO:16];
- (b) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-  
Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Glu-Leu-Cys-Glu-  
Leu-Pro-Pro-Asp-Thr-Gly-Pro-Cys-Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-  
25 Tyr-Asn-Pro-Asp-Glu-Gln-Lys-Cys-Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-  
Glu-Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-  
Cys-Ala-Ala [SEQ ID NO:18];

- (c) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-  
Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Asn-Phe-Cys-Lys-  
Leu-Pro-Ala-Glu-Thr-Gly-Arg-Cys-Asn-Ala-Lys-Ile-Pro-Arg-Phe-Tyr-Tyr-  
Asn-Pro-Arg-Gln-His-Gln-Cys-Ile-Glu-Phe-Leu-Tyr-Gly-Gly-Cys-Gly-  
5 Gly-Asn-Ala-Asn-Asn-Phe-Lys-Thr-Ile-Lys-Glu-Cys-Glu-Ser-Thr-Cys-  
Ala-Ala [SEQ ID NO:20];
- (d) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-  
Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-His-Pro-Lys-Phe-Cys-Glu-Leu-  
Pro-Ala-Glu-Thr-Gly-Ser-Cys-Lys-Gly-Asn-Val-Pro-Arg-Phe-Tyr-Tyr-  
10 Asn-Ala-Asp-His-His-Gln-Cys-Leu-Lys-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-  
Gly-Asn-Ala-Asn-Asn-Phe-Lys-Thr-Ile-Glu-Glu-Gly-Lys-Ser-Thr-Cys-  
Ala-Ala [SEQ ID NO:22];
- (e) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-  
Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-  
15 Leu-Leu-Pro-Asp-Thr-Gly-Ser-Cys-Glu-Asp-Phe-Thr-Gly-Ala-Phe-His-  
Tyr-Ser-Thr-Arg-Asp-Arg-Glu-Cys-Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-  
Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-  
Ala-Ala; [SEQ ID NO:24]; and
- (f) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-  
20 Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-  
Leu-Pro-Ala-Asp-Ile-Gly-Pro-Trp-Asp-Asp-Phe-Thr-Gly-Ala-Phe-His-Tyr-  
Ser-Pro-Arg-Glu-His-Glu-Cys-Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Lys-Gly-  
Asn-Ala-Asn-Asn-Phe-Asn-Thr-Gln-Glu-Gln-Cys-Glu-Ser-Thr-Cys-Ala-  
Ala; [SEQ ID NO:26].
- 25 41. An isolated polynucleotide encoding the polypeptide of claim 8.
42. An isolated polynucleotide selected from the group consisting of:-
- (a) AAGGACCGTCCGGATTTCTGTGAACTGCCTGCTGACACCGGACC

ATGTAGAGTCAGATTCCCATCCTTCTACTACAACCCAGATGAAAA  
AAAGTGCTAGAGTTTATTTATGGTGGATGCGAAGGGAATGCTAA  
CAATTTTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGCCT  
GA [SEQ ID NO:1];

5 (b) AAGGACCGTCCAGAGTTGTGTGAACTGCCTCCTGACACCGGACC  
ATGTAGAGTCAGATTCCCATCCTTCTACTACAACCCAGATGAACA  
AAAATGCCTAGAGTTTATTTATGGTGGATGCGAAGGGAATGCTA  
ACAATTTTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGCC  
TGA [SEQ ID NO:3];

10 (c) AAGGACCGTCCAAATTTCTGTAAACTGCCTGCTGAAACCGGACG  
ATGTAATGCCAAAATCCCACGCTTCTACTACAACCCACGTCAAC  
ATCAATGCATAGAGTTTCTCTATGGTGGATGCGGAGGGAATGCT  
ACAATTTTAAGACCATTAAGGAATGCGAAAGCACCTGTGCTGC  
ATGA [SEQ ID NO:5];

15 (d) AAGGACCATCCAAAATTCTGTGAACTCCCTGCTGAAACCGGATC  
ATGTAAAGGCAACGTCCCACGCTTCTACTACAACGCAGATCATC  
ATCAATGCCTAAAATTTATTTATGGTGGATGTGGAGGGAATGCTA  
ACAATTTTAAGACCATAGAGGAAGGCAAAGCACCTGTGCTGCC  
TGA [SEQ ID NO:7];

20 (e) AAGGACCGTCCAAAATTCTGTGAACTGCTTCCTGACACCGGATC  
ATGTGAAGACTTTACCGGAGCCTTCCACTACAGCACACGTGATC  
GTGAATGCATAGAGTTTATTTATGGTGGATGCGGAGGGAATGCT  
ACAATTTTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGC  
CTGA [SEQ ID NO:9];

25 (f) AAGGACCGTCCAAAGTTCTGTGAACTGCCTGCTGACATCGGACC  
ATGGGATGACTTTACCGGAGCCTTCCACTACAGCCCACGTGAAC  
ATGAATGCATAGAGTTTATTTATGGTGGATGCAAAGGGAATGCT

AACAACCTTTAATACCCAAGAGCAATGCGAAAGCACCTGTGCTGC  
CTGA [SEQ ID NO:11];

- (g) a polynucleotide fragment of any one of SEQ ID NOS 1, 3, 5, 7, 9 and 11,  
wherein said polynucleotide fragment encodes a biologically-active  
5 fragment of any one of SEQ ID NO:2, 4, 6, 8, 10 and 12; and  
(h) a polynucleotide homologue of any of the foregoing sequences.

43. The polynucleotide of claim 42 further comprising a nucleotide sequence  
encoding a leader peptide.

44. The polynucleotide of claim 43, wherein the nucleotide sequence comprises  
10 the sequence:-

ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCTCTG  
GGAGGTGCTGACCCCCGTCTCCAGC [SEQ ID NO:13] or a biologically  
active fragment thereof, or a polynucleotide homologue of these.

45. The polynucleotide of claim 43, wherein said polynucleotide is selected from  
15 the group consisting of:-

(a) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCTCT  
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCGGATTTC  
TGTGAACTGCCTGCTGACACCGGACCATGTAGAGTCAGATTCCC  
ATCCTTCTACTACAACCCAGATGAAAAAAAGTGCCTAGAGTTTAT  
20 TTATGGTGGATGCGAAGGGAATGCTAACAATTTTATCACCAAAG  
AGGAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:15];

(b) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCTCT  
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAGAGTTG  
TGTGAACTGCCTCCTGACACCGGACCATGTAGAGTCAGATTCCCA  
25 TCCTTCTACTACAACCCAGATGAACAAAAATGCCTAGAGTTTATT  
TATGGTGGATGCGAAGGGAATGCTAACAATTTTATCACCAAAGA  
GGAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:17];

- (c) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCCCTCT  
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAAATTTTCT  
TGTAAGCTGCCTGCTGAAACCGGACGATGTAATGCCAAAATCCC  
ACGCTTCTACTACAACCCACGTCAACATCAATGCATAGAGTTTCT  
5 CTATGGTGGATGCGGAGGGAATGCTAACAATTTTAAGACCATTA  
AGGAATGCGAAAGCACCTGTGCTGCATGA [SEQ ID NO:19];
- (d) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCCCTCT  
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCATCCAAAATTC  
TGTGAAGCTCCCTGCTGAAACCGGATCATGTAAAGGCAACGTCCC  
10 ACGCTTCTACTACAACGCAGATCATCATCAATGCCTAAAATTTAT  
TTATGGTGGATGTGGAGGGAATGCTAACAATTTTAAGACCATAG  
AGGAAGGCAAAAGCACCTGTGCTGCCTGA [SEQ ID NO:21];
- (e) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCCCTCT  
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAAAATTC  
15 TGTGAAGCTGCTTCCTGACACCGGATCATGTGAAGACTTTACCGGA  
GCCTTCCACTACAGCACACGTGATCGTGAATGCATAGAGTTTATT  
TATGGTGGATGCGGAGGGAATGCTAACAATTTTATCACCAAAGA  
GGAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:23];
- (f) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCCCTCT  
20 GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAAAGTTC  
TGTGAAGCTGCCTGCTGACATCGGACCATGGGATGACTTTACCGG  
AGCCTTCCACTACAGCCCACGTGAACATGAATGCATAGAGTTTAT  
TTATGGTGGATGCAAAGGGAATGCTAACAATTTAATACCCAAG  
AGCAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:25]; and
- 25 (g) GGAGCTTCATCATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGAC  
TCCTCACCCCTCTGGGAGGTGCTGACCCCCGTCTCCAGCAAGGACC  
GTCCAGAGTTGTGTGAAGCTGCCTCCTGACACCGGACCATGTAGA



GTCAGATCCCCATCCTTCTACTACAACCCAGATGAACAAAAATG  
CCTAGAGTTTATTTATGGTGGATGCGAAGGGAATGCTAACCAATT  
TTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGCCTGAATG  
AGGAGACCCTCCTGGATTGGATCGACAGTTCCAACCTTGACCCAA  
5 AGACCCTGCTTCTGCCCTGGACCACCCTGGACACCCTTCCCCCAA  
ACCCACCCCTGGACTAATTCCTTTTCTCTGCAATAAAGCTTTGGT  
TCCAGCT [SEQ ID NO:43]

46. A pharmaceutical composition for alleviating blood loss in a patient, said composition comprising the polypeptide of claim 8 and a pharmaceutically acceptable carrier.
- 10 47. A method for alleviating blood loss comprising the step of administering to a patient in need of such treatment a therapeutically effective dosage of the polypeptide of claim 8 in combination with a pharmaceutically acceptable carrier.
- 15 48. An anti-tumour agent comprising the polypeptide of claim 8 conjugated with an anti-fibrin antibody.